

A1 -
CULT.

- ?
- (c) determining a normalized amount of candidate compound in the at least one bile canaliculus, the amount of the candidate compound in the at least one bile canaliculus indicating the susceptibility of the candidate compound to biliary excretion.

A2

See
P. 10

13. (Once Amended) A method of screening a plurality of candidate compounds simultaneously *in vitro* for susceptibility to *in vivo* biliary excretion, the method comprising:

- (a) providing a plurality of cultures of hepatocytes, wherein each culture of hepatocytes comprises at least one bile canaliculus;
- (b) exposing a different candidate compound within the plurality of candidate compounds to each culture within the plurality of cultures; and
- (c) determining a normalized amount of candidate compound in the at least one bile canaliculus, the amount of the candidate compound in the at least one bile canaliculus indicating the susceptibility of the candidate compound to biliary excretion.

Sub
B1

A3

25. (Once Amended) A method of screening a candidate compound *in vitro* for susceptibility to *in vivo* biliary excretion, the method comprising the steps of:

- (a) providing a culture of hepatocytes, the culture comprising at least one bile canaliculus;
- (b) exposing a candidate compound and a pre-selected amount of a marker compound to the culture for a time sufficient to allow uptake;
- (c) washing the culture; and
- (d) detecting an amount of marker compound present in the at least one bile canaliculus in the culture to evaluate uptake and excretion competition between the candidate compound and the marker compound, the presence or the absence of a reduced amount of the marker compound as compared to the pre-selected amount of marker compound indicating the susceptibility of the candidate compound to biliary excretion.

A4

39. (Once Amended) A method of screening a candidate compound *in vitro* for susceptibility to *in vivo* biliary excretion, the method comprising the steps of:

- (a) establishing first and second cultures of hepatocytes, each culture comprising at least one bile canaliculus, the first culture having intact bile canaliculi and the second culture having disrupted bile canaliculi;
- (b) exposing a candidate compound to the first culture and to the second culture for a time sufficient to allow uptake of the candidate compound;
- (c) washing and then lysing the first and second cultures;
- (d) determining a normalized amount of candidate compound present in a lysate obtained from each culture in step (c); and
- (e) calculating a biliary clearance value by subtracting: (the normalized amount of the candidate compound present in the lysate of the second culture, as measured in step (d), from the normalized amount of the candidate compound present in the lysate of the first culture, as measured in step (d)) and dividing by (the time of step (b) that the candidate compound was exposed to the hepatocytes multiplied by an initial concentration of the candidate compound in a buffer medium), the calculated biliary clearance value indicating the susceptibility of the candidate compound to biliary excretion.

A5

52. (Once Amended) A method of screening a metabolite of a candidate parent compound *in vitro* for susceptibility to *in vivo* biliary excretion, the method comprising the steps of:

- (a) establishing a first set and second set of two cultures of hepatocytes, each culture comprising at least one bile canaliculus, a first culture within each set having intact bile canaliculi and a second culture within each set having disrupted bile canaliculi;

A5
CONT.

- (b) exposing a candidate parent compound to the first culture and to the second culture of each set for a time sufficient to allow uptake of the candidate parent compound;
- (c) inducing Phase I, Phase II, or transport metabolic enzyme activity, or combinations thereof, in the hepatocytes of the first set of cultures;
- (d) washing and lysing the first and second cultures of each set;
- (e) determining a normalized amount of candidate parent compound present in a lysate obtained from each culture in step (d);
- (f) determining a normalized amount of the metabolite of the candidate parent compound present in a lysate obtained from each culture in step (d);
- (g) calculating a biliary clearance value for the candidate parent compound by subtracting (the normalized amount of the candidate parent compound present in the lysate of the second culture, as measured in step (e), from the normalized amount of the candidate parent compound present in the lysate of the first culture, as measured in step (e)) and dividing by (the time of step (b) that the candidate parent compound was exposed to the hepatocytes multiplied by an initial concentration of the candidate compound in a buffer medium), the calculated biliary clearance value indicating the susceptibility of the candidate parent compound to biliary excretion; and
- (h) calculating a biliary clearance value for the metabolite of the candidate parent compound by subtracting (the normalized amount of the metabolite of the candidate parent compound present in the lysate of the second culture, as measured in step (f), from the normalized amount of the metabolite of the candidate parent compound present in the lysate of the first culture, as measured in step (f)) and dividing by (the time of step (b) that the candidate parent compound was exposed to the hepatocytes multiplied by an initial concentration of the candidate compound in a buffer